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(54) Title: MICROBIAL PRODUCTION OF CIS-DIHYDRODIOL AND PHENOL DERIVATIVES OF BENZOCYCLOBUTENE

(57) Abstract

A process for microbial conversion of benzocyclobutene to the corresponding 4,5-dihydrodiol followed by acid catalyzed dehydration to 4-hydroxybenzocyclobutene.

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TITLE

MICROBIAL PRODUCTION OF CIS-DIHYDRODIOL AND PHENOL DERIVATIVES OF BENZOCYCLOBUTENE

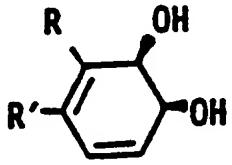
BACKGROUND OF THE INVENTION

1. Field of the Invention:

The present invention relates to the bioconversion of benzocyclobutene (BCB) to the 4,5-cis-dihydrodiol compound and the subsequent acid-catalyzed dehydration to form the 4-hydroxybenzocyclobutene compound. These novel compounds have utility as intermediates for the production of polymers.

2. Description of the Related Art:

Formation of cis-dihydrodiols from various aromatic hydrocarbons by bacteria has been described by D. T. Gibson et al., Biochemistry, vol. 9, No. 7, 1973, p. 1626+ and p. 1631+ and vol. 12, No.8, 1973, p. 1520+. A cis-dihydrodiol intermediate has been found to be a common metabolite in the bacterial degradation of a variety of aromatic hydrocarbons, including benzene, toluene, naphthalene, biphenyl, ethylbenzene, benzoic acid, phthalic acid, anthracene and phenanthrene. U.S. Patent No. 4,508,822 discloses the preparation of dihydrodiols of the general formula:



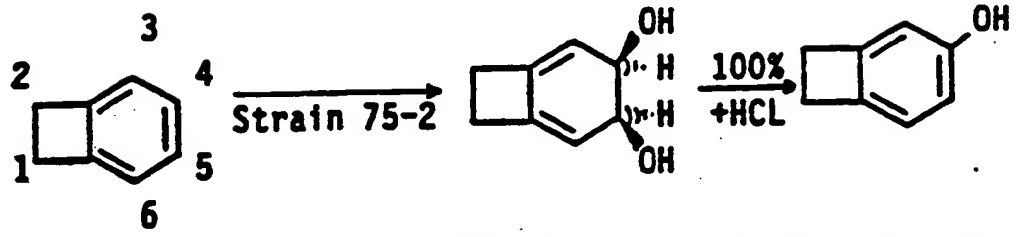
where R and R^1 are substituents which may be the same or different, such as halogen, alkyl, and the like. Generally such dihydrodiols are of the 2,3-dihydrodiol configuration. That is, the hydroxy groups are introduced directly adjacent to the ring substituent R. The only known exception to this general rule is the 4,5-dihydrodiol formed by some bacteria in the degradation of phthalic acid.

U.S. Patent 4,520,103 describes the formation of the 2,3-dihydrodiol of indole as an intermediate in the production of indigo.

SUMMARY OF THE INVENTION

The present invention relates to the formation of a dihydrodiol resulting from bacterial bioconversion of the aromatic hydrocarbon benzocyclobutene. Mutant strains of Rhodococcus organisms capable of converting benzocyclobutene to the 4,5-dihydrodiol have been developed. The growth of the mutant strain in the presence of benzocyclobutene results in the production of the 4,5-dihydrodiol intermediate of benzocyclobutene. Acid-catalyzed dehydration of the 4,5-dihydrodiol compound results in formation of 4-hydroxybenzocyclobutene. The corresponding sequential reactions are outlined below.

4-Hydroxybenzocyclobutene



4,5-Dihydrodiol 4-Hydroxybenzocyclobutene

DETAILED DESCRIPTION OF THE INVENTION

Organisms capable of growth on a variety of aromatic hydrocarbons such as benzene, toluene, ethylbenzene and o-xylene were isolated from the environment by selective culture. Certain of the resulting isolates were found to partially metabolize benzocyclobutene to a mixture of dead-end metabolites, but were not able to grow on benzocyclobutene. Mutants lacking a functional diol dehydrogenase were obtained by mutagenesis with N-methyl-N-nitro-N-nitrosoguanidine, followed by ampicillin/cycloserine enrichment for mutants unable to grow on toluene. Diol dehydrogenase deficient mutants were identified by the accumulation of dihydrodiols upon exposure to various aromatic hydrocarbons.

The Mutant, 75-2, derived from a Rhodococcus isolate 75 WT, converts benzocyclobutene to the corresponding 4,5-dihydrodiol compound. The dihydrodiol at a concentration of two hundred to four thousand parts per million in aqueous solution is dehydrated by addition of a mineral acid such as hydrochloric acid or sulfuric acid to a concentration

of 0.1 N to 8 N, preferably 1.0 to 5N at a temperature of 20° to 50°C for 15 minutes to 20 hours, preferably 1 to 10 hours. The resulting phenols can be recovered such as by extraction with water immiscible, polar organic solvents, such as ethyl acetate, methyl ethyl ketone, or the like. Generally over 95% of the recovered phenols are 4-hydroxybenzocyclobutene, with the balance 3-hydroxybenzocyclobutene. The 3-hydroxybenzocyclobutene results from a low level of hydroxyl migration during the dehydration reaction.

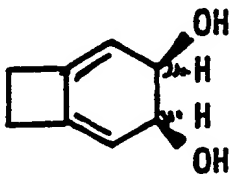
Example

Rhodoccus strain 75-2 American Type Culture Collection (ATCC) 55201 is grown in baffled 125 ml Erlenmeyer flasks on a minimal salts medium with succinate at 1.0 wt % . Benzocyclobutene is supplied as a vapor to the culture. After 24 hours incubation on a rotary shaker at 150 rpm and 30°C, the culture is acidified with HCl to a concentration of 1.0 N, and held at room temperature for 4 hours. The broth was then extracted with an equal volume of ethyl acetate, and analyzed for phenols by gas chromatography. The 4-Hydroxybenzocyclobutene was present at 235 ppm, the 3-hydroxybenzocyclobutene at 7 ppm.

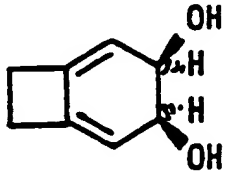
CLAIMS

1. 4-Hydroxybenzocyclobutene.

2. A dihydrodiol of the formula



3. A process for production of a dihydrodiol compound of the formula



comprising growing a mutant strain of Rhodococcus in a growth medium at 25° to 35°C and at a pH in the range of 6 to 8, in the presence of oxygen or an oxygen containing gas wherein benzocyclobutene is supplied to the growing mutant strain.

4. The process of claim 3 wherein the mutant strain is a strain of Rhodococcus WT.

5. The process of claim 4 wherein the dihydrodiol compound is treated at 20° to 50°C with an aqueous acid solution containing 0.1 to 8 N mineral acid for 15 minutes to 20 hours to form 4-hydroxybenzocyclobutene.

6. The process of claim 5 wherein the 4-hydroxybenzocyclobutene is extracted from the acidified solution with a water immiscible, polar organic solvent.

7. The process of claim 6 wherein the strain is Rhodococcus ATCC 55201.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 92/09214

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C12P 7/22, C07C 39/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C12P, C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Chemical Society Journal. Perkin transactions I, Volume 8, 1980, Omar Abou-Teim et al., "Benzocyclobutenes. Part 5.1 Synthesis of 4-Hydroxy-, 4,5-Dihydroxy-, and 3, 6-Dihydroxy-benzocyclobutene-1,2-dione (Benzologues of Semisquaric and Squaric Acid)", page 1841 - page 1846, see example 3, p 1841	1-7
A	J.Org.Chem., Volume 47, No 20, 1982, Michael S. South et al., "Practical Multigram Syntheses of Benzocyclobutenediones", page 3816 - page 3821, see p. 3816	1-7

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 February 1993	15 MAR 1993
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INTERNATIONAL SEARCH REPORT

International application N .
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, A2, 0158424 (DIRECTOR-GENERAL OF THE AGENCY OF INDUSTRIAL SCIENCE AND TECHNOLOGY), 16 October 1985 (16.10.85)	3-7

INTERNATIONAL SEARCH REPORT

Information on patent family members

29/01/93

International application No.
PCT/US 92/09214

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0158424	16/10/85	JP-C- 1435684	25/04/88
		JP-A- 60210991	23/10/85
		JP-B- 62046157	30/09/87
		US-A- 4824780	25/04/89
		JP-C- 1435685	25/04/88
		JP-A- 60210992	23/10/85
		JP-B- 62046158	30/09/87